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Dr. Barbara S. Shane
NTP Executive Secretary
National Institute of Environmental Health Sciences
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Dear Dr. Shane:

The Dow Chemical Company has conducted a limited review of the draft NTP report (NTP TR534) entitled 'Toxicology and Carcinogenesis Studies of Divinylbenzene-HP (CAS No. 1321-74-0) in F344/N Rats and B6C3F1 Mice (Inhalation)' and offers the following comments for your consideration.

While conservative vis-à-vis inclusion of the non-dose-related, non-statistically significant glial tumor data from the male rat as part of the supporting evidence, the conclusions of 'Equivocal evidence' for male rats and female mice, and 'No evidence' for female rats and male mice for divinylbenzene (DVB) are acceptable and supported by the majority of the data described in the draft report.

There is other available information that could be added to the report to further clarify and/or provide perspective on several different aspects, as described below.

1. Additional information is available to further clarify the kidney lesions identified in male rats. In particular, the following points should be considered for inclusion:

   1a. Published information on the step-sectioning of kidney from control rats is available (Eustis *et al*., 1994[1]), and this data or other data from step-sectioned kidney of control male rats should be included for comparison. Eustis *et al*. report that tubular hyperplasia or adenomas are much more common using step sectioning with incidence rates of 9.40% (range 0-20%) for hyperplasia and 4.47% (0-16%) for adenoma in control male F344 rats *vs*. 3.39% (0-8%) and 0.62% (0-2%), respectively, using conventional single sections plus grossly observable lesions. Thus, these small adenomas are fairly common in untreated male rats, and the adenomas identified in the current study, 2 and 1 at 200 and 400 ppm, respectively, are not unusual. This should be mentioned in the report.

[1] Eustis *et al*. (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol. Pathol*. **22**: 457-472.

1b. Step-sectioning was required to identify the presence of additional kidney adenomas, which would indicate that the adenomas identified were quite small, and not identified grossly. We recommend you consider describing these as 'microadenomas'.

1c. Male rat renal tumors have been associated with the accumulation of alpha-2□-globulin and with the age-related Chronic Progressive Nephropathy (CPN). There was no mention of either of these as potential mechanisms/modes-of-action for the renal tumors identified in the draft report. The report notes increased incidence of CPN in 400 ppm male rats, although the mean severity was similar, and equivocally increased incidence and mean severity in males given 200 ppm. However, individual animal non-neoplastic lesions were not presented and we were unable to assess any possible correlation between CPN, hyperplasia and tumors. It would be helpful to include consideration of theses effects and clarify any possible association (or lack thereof) in the report.

2. Additional information is available to further clarify the brain lesions identified in male rats. In particular, the following points should be considered for inclusion in the report:

2a. The lack of any tumors at the high dose suggests that the occurrence of glial tumors is spurious. In addition, the glial tumors identified were of late onset. We recommend that the abstract note the late-onset of the glial tumors, and that their incidence was not dose-related, in addition to the lack of statistical significance.

2b. There are published data on historical control incidences from feeding and chamber studies (Haseman *et al*., 1998[2]), which reported the following incidences for control male rats: gliomas (0-4%), astrocytomas (0-4%) and oligodendrogliomas (0-2%). These values indicate that the incidence of astrocytomas and oligodendrogliomas, combined, in the current study of 2% and 6% for 100 and 200 ppm DVB, respectively, are likely within the historical control incidence.

2c. These tumors were identified either late (100 ppm: d582) or only at terminal sacrifice (200 ppm: d729, 730, and 731). This late onset should be noted in the report.

3. Additional information is available to further clarify the alveolar/bronchiolar (A/B) lesions identified in male and female mice, including the non-dose-related and non-statistically significant increased incidence of A/B adenomas and carcinomas, combined, for female mice. In particular, the following points should be considered for inclusion in the report:

3a. There are published data on historical control tumor incidences from feeding and chamber studies (Haseman *et al*., 1998[3]), which report the following incidences for control female mice: A/B adenoma (0-24%), and A/B carcinomas (0-12%). These values would indicate that the current study incidence of A/B adenomas and carcinomas, combined, of 24%, 16%, and 27% at 10, 30, and 100 ppm, respectively, are likely within the historical control incidence.

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[2] Haseman et al. (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program Update. *Toxicol. Pathol*. **26**: 428-441.

[3] *Ibid.*

3b. Most of these tumors were identified only at terminal sacrifice, while the few that were identified earlier were still late (day 697 in the 100 ppm group). This late-onset should be noted in the report.

3c. The atypical bronchiolar hyperplasia described for both female and male mice was compared with effects from styrene (p 80 draft report). Published results for styrene indicate that this mouse-specific lung effect is due to Clara cell-specific metabolism of styrene by cytochrome P450 2F, as is described by Cruzan *et al*. (2002[4]). The lack of lung effects in rats of either sex exposed to DVB further supports this as a species-specific effect and is similar to that noted with styrene. Furthermore, the published reference demonstrates that human lung tissue was at least 100-fold less effective at styrene metabolism to styrene oxide, indicating that the postulated mode-of-action for styrene A/B effects is not relevant to humans. This should be mentioned in the report.

3d. The lack of dose-response in the A/B tumor incidence in female mice should be included in the abstract.

4. Additional information is available to further clarify the nasal lesions identified in rats and mice. In particular, the following point should be considered for inclusion in the report:

4a. As noted in the report (p.91), the nasal lesions found in both rat and mice were attributed to a metabolite of styrene rather than the parent compound. In this same reference, Green et al. (2001b) could not detect the metabolism of styrene to styrene oxide in human nasal tissue *in vitro* and concluded that styrene is unlikely to be toxic to human nasal epithelium. This information should be mentioned in the report.

In summary, although they are conservative, Dow supports the conclusions of 'Equivocal evidence' for male rat and female mouse, and 'No evidence' for female rat and male mouse, as presented in the draft report. We believe that including the points made above in the final report would add useful perspective to the DVB data presented by NTP and strengthen the conclusions noted above.

If you have any questions on the comments provided or wish to discuss any points further, please contact me at the following address: cldeford@dow.com

Sincerely,

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[4] Cruzan et al. (2002). Styrene respiratory tract toxicity and mouse lung tumors are mediated by CYP2F-generated metabolites. *Reg. Toxicol. Pharmacol*. **35**: 308-319.